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IN VIVO ANTIMALARIAL EFFICACY OF THE COMBINED METHANOL LEAF EXTRACTS OF *Dacryodes edulis* AND *Funtumia elastica* IN ALBINO MICE INFECTED WITH CHLOROQUINE SENSITIVE *Plasmodium berghei* (NK65)

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ABSTRACT
Malaria is a major public health problem, and the alarming speed of drug resistance and limited number of effective drugs now available underlines how important it is to discover new antimalarial compounds. This work investigated the antimalarial efficacy of the combined methanol leaf extracts of Dacryodes edulis and Funtumia elastica. The two plant leaves were extracted separately with methanol and tested for their antimalarial activity against chloroquine (CQ) – sensitive strain of Plasmodium berghei (NK-65) infected mice. Through oral administration of the extracts (250, 500, 1000 mg/kg bwt), their antiplasmodial activities were screened using the 5-day curative test. The combined leaf extracts showed moderate antiplasmodial effects at the different doses. Hematological indices were normalized in the groups of mice infected and treated with the combined leaf extracts. The result suggests that the combined methanol leaf extracts of Dacryodes edulis and Funtumia elastica possess moderate antiplasmodial activity. The study justifies local claims on the efficacy of these plant leaves in the treatment of malaria.

KEYWORDS: *Malaria, Plasmodium beghei, Dacryodes edulis, Funtumia elastica, antiplasmodial*

INTRODUCTION

Malaria caused by *Plasmodium* parasites, is a major global health burden, with an estimated 229 million cases and 409,000 deaths in 2019 alone (WHO, 2020). *Plasmodium falciparum* causes almost half of all malaria cases, and the majority of deaths are children in sub-Saharan Africa; *Plasmodium vivax* accounts for 65% of malaria cases in Asia and South America (WHO, 2020).

Resistance to anti-malarial medicines pose a serious threat to the global efforts to control and annihilate malaria. Recently, resistance to artemisinin has been reported in the form of delayed parasite clearance in Southeast Asia, posing a threat to the current first-line artemisinin-based combination therapy (Fairhurst and Dondorp, 2016; Zhao *et al.,* 2019). For *P. vivax*, the spread of resistance to chloroquine, primaquine, mefloquine, and SP has been reported in various

regions of the world (Benavente *et al.,* 2017; Ngassa Mbenda *et al.,* 2020). The underlying mutations causing resistance for *P. vivax* are less well defined than for *P. falciparum* (Benavente *et al.,* 2017; Ngassa Mbenda *et al.,* 2020; Diez Benavente *et al.,* 2021). Protecting and monitoring the efficacy of antimalarial treatments is a top priority for malaria endemic countries.

Several recent publications have even reported that *Plasmodium falciparum* has also shown an increase in resistance to artemisinin based antimalarial (Htut, 2009). Therefore, it is urgent to continue efforts of searching for new and more effective drugs. This prompted the researchers to find other alternative approach, such as evaluating medicinal plants (Christian *et al.,* 2015). Research on medicinal plants of various traditional medicine systems could provide useful leads for the development of important active compounds (Lemma *et al.,* 2017). In addition, exploration of traditional medicine for malaria treatment is useful to design strategies that can be further developed to support a more effective and culturally acceptable malaria eliminating program (Serengbe *et al.,* 2015).

Dacryodes edulis common names in Nigera include native pear, *Ube*, *Olumu* and *Orunmwun*. It found in equatorial and humid tropic climates and originates from Central Africa and Gulf of Guinea area (Ayuk *et al.*, 1999). The tree is also a source of many herbal medicines. It has long been used in the traditional medicine of some African countries to treat various ailments such as wounds, skin diseases, dysentery, and fever. The extracts and secondary metabolites have been found to show antimicrobial and antioxidant activities. (Omonhinmin and Agbara, 2013).

Funtumia elastica (Lagos silk rubber) is a medium-sized African rubber tree with glossy leaves, milky sap, and long woody seedpods. The bark is portion of the plant known for medicinal effect. *Funtumia elastica* has important antioxidant, antifungal, anti-inflammatory, and antibiotic properties (Bogne *et al.,* 2007).

The use of a combination of the two extracts for this study was due to the emergence and spread of malaria parasite with resistance to antimalarial drugs. This study investigated the anti-plasmodial effects of the combined methanol leaf extracts of *Dacryodes edulis* and *Funtumia elastica* against *Plasmodium berghei* NK65 infected mice.

MATERIALS AND METHOD

Plant Collection and Authentication

The leaves of *Dacryodes edulis* and *Funtumia elastica* were obtained in the month of November 2020 at Benin City, Edo state, Nigeria. The identification and authentication were carried out in the Department of Pharmacognosy by the University Herbarium, at the University of Benin, Benin City, Edo State.

Preparation of Plant Extract

Fresh leaves of *Dacryodes edulis* and *Funtumia elastica* were air-dried in the laboratory. The dried pieces were then hand crushed. 500g of dried powdered form of the plant material was extracted by soaking in methanol for 72hours. The extract was concentrated using a rotary evaporator. The extracts were then stored in well-sealed containers and kept in a refrigerator at 4°C to protect them from light and

moisture for subsequent use (Sutharson *et al*., 2007). Thereafter, the two prepared extracts were mixed together in the ratio of 1:1 to form a combined therapy.

Preparation of Stock Solution

0.5g of the combined extract was weighed from each of the concentrated extracts into a conical flask and dissolved with 50 ml of distilled water to make stock solutions.

Experimental Animals

The animals used for this study were healthy Swiss albino male mice weighing between 18-30g. They were obtained from the Animal faculty, Center of the Department of Pharmacology and Toxicology, University of Ibadan, Ibadan, Oyo State, Nigeria. The mice were housed under standard conditions at temperature 25ºC, relative humidity 70% and at 12 hour day/night cycles. They had free access to grower mash and water. The animals were allowed to acclimatize for one week before commencing the experiment. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animals' use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (Ernest *et al*. 1993).

Acute Toxicity

Acute toxicity was conducted to determine the range of lethal doses (LD_{50}) in the animals. The study mice were fasted overnight and randomly assigned into 6 groups of 2 animals each. Methanol extract of *Dacryodes edulis* and *Funtumia elastica* was orally administered separately to each animal at doses 10,100, 1000, 1600, 2900 and 5000mg/kg. The first group of 2 mice was orally administered with the vehicle Carboxyl Methyl Cellulose (CMC) and served as the positive control. The test material was prepared in suspension for oral administration. Each animal was observed for signs of acute toxicity, poisoning symptoms and mortality for a period of 72 hours.

Inoculation of Animals

The Chloroquine - sensitive *Plasmodium berghei* infected mice were obtained from Nigeria Institute of Medical Research (NIMR) Lagos, Nigeria and kept at the animal house in the Department of Biochemistry, Benson Idahosa University, Benin City, Edo State, Nigeria. 100µL of Parasitized Erythrocytes were obtained from donor-infected mice by cardiac puncture and made up to 3mL with normal saline. The animals were inoculated intra-peritoneal with 0.2 mL of the blood containing about 10^7 infected erythrocytes.

Evaluation of Antiplasmodial Activity of Extract (Curative Test)

Peters and Ryley's method (1970) with slight modification was used in the evaluation of curative activity on established infection. On the first day (day 0), standard inoculum of 10^7 *Plasmodium berghei* NK65 infected erythrocytes was injected intraperitoneally into the mice. After 72h post-inoculation, there was a confirmation of parasitemia in the infected groups except the mice in the control group, the animals were randomly divided into 6 groups of 8 mice each. Group A (normal control), Group B (infected but treated with standard drug control group) received 5mg/kg body weight of Chloroquine daily for 5 days. Group C (infected but not treated control group) were

administered appropriate volume of 0.5ml of carboxyl methyl cellulose (CMC). Groups (D, E and F - All infected) were administered orally with 0.5ml of 250, 500 and 1000 mg/kg body weight respectively of the combined leaf extract of *Dacryodes edulis* and *Funtumia elastica* for five days. The oral administration was done with the aid of a stainless metallic feeding cannula.

Collection of Samples

Animals were fasted overnight. The animals were placed under anaesthesia using chloroform and sacrificed by cervical dissection on 'day' 6 of the experiment, samples were then collected.

Collection of Blood and Serum Samples

Blood was collected from the abdominal aorta and the heart via a syringe into plain sterile bottles (for serum), EDTA tubes (for hematological analysis). Blood samples inside the plain tables were allowed to stand for one hour to clot and centrifuged at 3000rpm for 10 minutes at room temperature to obtain serum.

Haematological Assay

Standard haematological procedures of Dacie and Lewis were used for haematological analysis for packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and platelet.

Statistical Analysis

Values were expressed as mean ± SEM. Data from the test were compared with their respective controls using ANOVA and differences at $p < 0.05$ were considered significant.

RESULTS

Acute Toxicity

The acute toxicity results for the extracts indicate that none of the extracts caused mortality even up to 5000 mg/kg bwt. The results support the safe use of *Dacryodes edulis* and *Funtumia elastica* in traditional medicine.

Results for Curative Test

The result from the curative test was subjected to statistical analysis. There was a statistical difference between the infected but not treated control groups from other groups. From Figure 1 below, we understand that the infected control has the highest parasitemia count as against the group of mice treated for five days with different concentration of the combined extract of *D. edulis,* and *F.elastica*. But still, the mice group treated with chloroquine has almost same parasite count similar to the combined extract of DE+FE (1000mg/kg).

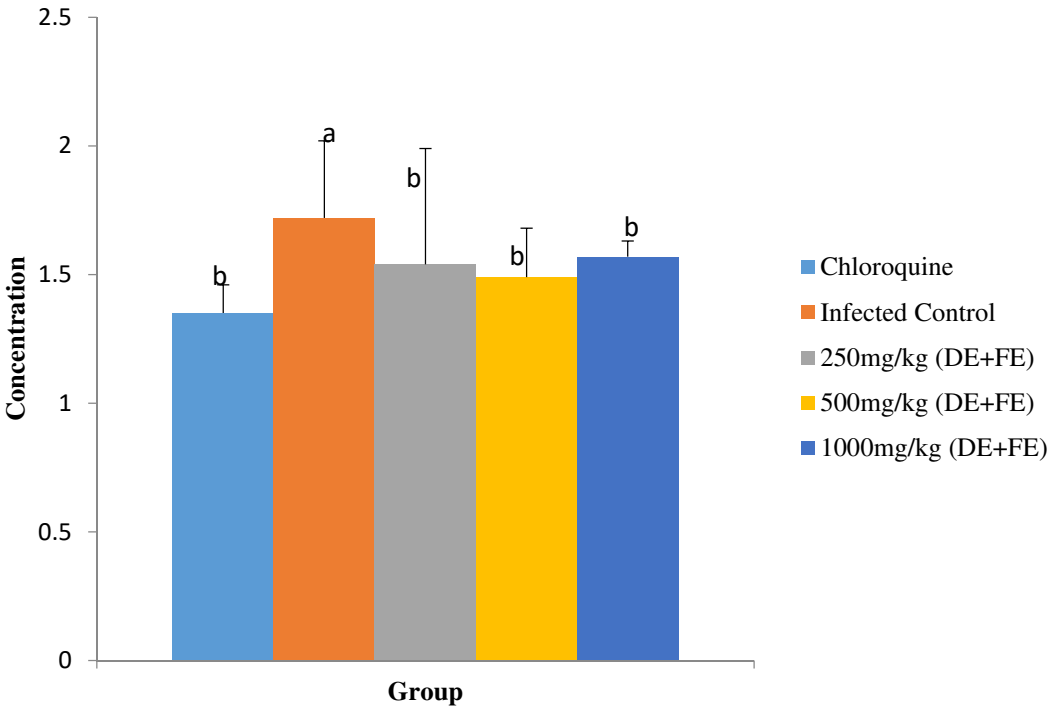

Fig. 1: Percentage Parasitaemia of *P. berghei* infected mice treated with the combined extracts.

Each bars are represented as mean ± SEM. Bars not sharing a common alphabet differ significantly at $p < 0.05$.

The effect of combined leaf extracts on haematological indices of P. berghei infected mice

The table below shows the effect of the combined extract on haematological indices of *P.berghei* infected mice. The infected mice group has high WBC count which indicates immunity to the parasite. The infected mice group has low RBC count and Hb concentration which signify ability to transport oxygen from the lungs to the tissues. But as for the groups treated with the combined extract, there is a normal count of platelet. Also, the PCV of the treated mice tends to increase against the infected mice.

Table 1: Effect of the combined leaf extracts on Haematological Indices of *P. berghei* infected Mice

Parameters	Normal Control	Chloroquine	Infected Control	250mg/kg (DE+FE)	500mg/kg (DE+FE)	1000mg/kg (DE+FE)
PCV (%)	30.00 $^{a\,b}$	33.50 ^{b}	22.00 ^{a}	29.00 $^{a\,b}$	27.0 $^{a\,b}$	33.50 ^{b}
Hb (g/dl)	10.05 ^{ab}	11.00 ^{b}	7.33 ^{a}	9.55 $^{a\,b}$	9.00$^{a\,b}$	11.20 ^{b}
WBC (10^{3}/µL)	15.95 ^{a}	19.7 $^{a\,b}$	28.67 ^{b}	15.70 ^{a}	19.35 $^{a\,b}$	18.90 $^{a\,b}$
RBC (10^{6}/µL)	3.70 $^{a\,b}$	4.15 ^{b}	2.90 ^{a}	3.75 $^{a\,b}$	3.35 $^{a\,b}$	4.20 ^{b}
MCH (pg)	81.50 ^{b}	81.00 ^{b}	74.67 ^{b}	3.75 ^{a}	80.50 ^{b}	80.00 ^{b}
MCV (fl)	27.00 ^{a}	26.50 ^{a}	25.0 ^{a}	25.5 ^{a}	26.5 ^{a}	27.50 ^{a}
MCHC (g/dl)	33.45 ^{a}	32.80 ^{a}	33.50 ^{a}	32.90 ^{a}	33.35 ^{a}	33.95 ^{a}
Platelets (10^{3}/µL)	392.00 ^{a}	356.0 ^{a}	312.70 ^{a}	344.00 ^{a}	358.00 ^{a}	388.50 ^{a}
Neutrophil (%)	29.50 ^{a}	47.00 ^{a}	45.00 $^{a\,b}$	39.50 $^{a\,b}$	45.50 $^{a\,b}$	35.50 $^{a\,b}$
Lymphocytes (%)	70.00 ^{b}	52.0 $^{a\,b}$	51.67 $^{a\,b}$	59.00 $^{a\,b}$	53.50 $^{a\,b}$	62.50 $^{a\,b}$
Monocytes (%)	0.00 ^{a}	0.50 ^{a}	0.67 ^{a}	1.00 ^{a}	1.00 ^{a}	0.50 ^{a}
Eosinophil (%)	0.00 ^{a}	0.50 ^{a}	1.00 ^{a}	0.50 ^{a}	1.00 ^{a}	1.50 ^{a}
Basophil (%)	0.00	0.00	0.00	0.00	0.00	0.00

Values are expressed as mean ± SEM. Values in same column with different alphabet are significantly different ($p < 0.05$).

DISCUSSION

Studies of the antiplasmodial effect of the combined methanol extracts of *Dacryodes edulis* and *Funtumia elastica* were carried out on albino mice experimentally infected with *P.berghei*. The choice of these plants was based on previous reports of their antiplasmodial property. The results of this study show that the combined extract has a moderate antimalarial property. The antiplasmodial activity of the combined extract could be attributed to the presence of the phytochemical compounds (Bassey *et al.,* 2009). Saganuwan *et al.* (2011) reported that plants which contain many phytochemicals with biological activities like alkaloids and flavonoids could serve as sources of antimalarial drugs. This property of the plants has been implicated in creation of an intracellular environment that is unfavourable to plasmodial growth (Alli *et al.,* 2011). This suggested that the antiplasmodial properties of the combined extract could be based on the antioxidant and effects of these phytochemicals (Ayoola *et al.,* 2008).

The infected control group observed a high percentage of parasitemia in *P.berghei* infected mice after five days and death of infected mice after seven days of inoculation. Hence, high level of parasitaemia is an important feature of *Plasmodium* infection which could result in severe anaemia and death of infected animals.

Plasmodium berghei parasites are used in predicting treatment outcomes of any suspected antimalarial agent. Due to its high sensitivity to chloroquine it is the appropriate parasite for this study (Peter and Anatoli, 1998; David *et al*., 2004). *Plasmodium berghei* has been used in studying the activity of potential antimalarial in mice (Pedronic *et al*., 2006). It produces diseases similar to those of human plasmodium infection (Kumar *et al*., 2006). There were significant decrease ($p < 0.05$) in parasite density in the

treated group compared to the untreated group (PnT). Kiseko *et al*. (2000) showed that when a standard anti-malarial drug is used in mice infected with *P. berghei*, it suppresses the parasiteamia to a non-detectable level which is in line with this study.The observed antiplasmodial activity of the extract is consistent with the traditional use of this plant as an herbal medication against malaria. The plant extract may prevent the detoxification of free oxidized heme, one of the by-products of haemoglobin degradation by intercalating with the iron-carboxylate bond which links the heme units of malaria pigment (hemozoin) thereby inhibiting polymerization (Becker *et al*., 2004; Ravikumar *et al*., 2012)

Studies have showed that hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Eluwa, 2011 and Ohaeri, 2011). WBC functions in the body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995).The results from this study show that the most significant changes in the hematological profiles were evident as parasitaemia bouts increases. Hematological profiles were normalized in the groups of mice infected and treated with the combined leaf extracts of *D. edulis* and *F. elastica*. The extracts prevented a drastic reduction in RBC and HGB values which is noticeable during malaria infections (Aleksandro *et al*., 2009). In addition, there was a gradual decline in WBC counts in the different experimental groups of infected and treated mice at a dose of 250, 500, and 1000mg/kg body weight. There were significant increases ($p < 0.05$) in the WBC count in parasitized not treated (PnT) group compared with all the groups treated with *D. edulis* and *Funtumia elastica* extract. Leukocytosis observed in PnT group may be due to bone marrow tumor, leukemia, tissue damage, and inflammatory disease of the mice infected with *P. berghei NK65*. This observation corroborates with previous reports that fluctuations of WBC counts are reflectors of persistent underlying infections (Ngotho *et al*., 2011).

The present study suggests moderate antiplasmodial activity of the combined methanol leaf extracts of *D. edulis* and *F. elastica*. The results therefore justifies the traditional use of *D. edulis* and *F. elastica* leaves in the local treatment of malaria.

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